

MtZIP6 is a novel metal transporter required for symbiotic nitrogen fixation in nodules of *Medicago truncatula* plants.

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Symbiotic nitrogen fixation (SNF) carried out by the interaction rhizobia-legumes takes place in legume root nodules. Many of the enzymes involved in SNF are metalloproteins that obtain their metal cofactor from the host plant. Metals reach the nodule through the vasculature, where they are released in the apoplast on the infection/differentiation zone (zone II) of the nodule (Rodríguez-Haas et al., 2013). From there, these oligonutrients have to cross a number of membranes to be used for metalloprotein synthesis (plasma membrane, endoplasmic reticulum, symbiosomes,...). Although several proteins have been suggested to mediate metal transport to the endosymbiotic nitrogen-fixing rhizobia (bacteroids), very little is known about transporters that mediate metal uptake from the apoplast. Recently, we have identified MtNramp1, the first iron transporter to mediate this uptake (Tejada-Jiménez et al., 2015). However, other transporters must mediate zinc, manganese or copper uptake from the nodule apoplast.

Transcriptomic studies in *Medicago truncatula* revealed that MtZIP6, a ZIP family member, had a maximum of expression in the nodule. ZIP6 promoter::GUS fusions showed that MtZIP6 expression was confined to the nodule zone II, the region where metals have to be incorporated from the apoplast. These results were also validated by immunohistochemistry in nodule sections expressing MtZIP6 bound to 3xHA epitopes under the MtZIP6 promoter. This experiment showed that MtZIP6 is very likely localized in the plasma membrane and confirmed its expression in zone II of the nodule. Expression of MtZIP6 in *Saccharomyces cerevisiae* metal transport mutants showed MtZIP6 as a divalent metal importer, capable of transporting zinc, manganese, or iron. Loss of MtZIP6 function in *M. truncatula* knocked-down plants revealed a reduced plant and nodule size, and a reduced nitrogenase activity in comparison to control plants. Altogether these results suggest that MtZIP6 is an important element in the process of symbiotic nitrogen fixation by its ability to transport metals through the plasma membrane of the nodule cells.

Rodríguez-Haas,B, et al (2013). Metallomics 5: Page 1247-1253

Tejada-Jimenez M, et al (2015) Plant Physiol. Doi:10.1104/pp.114.254672

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